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# TECHNICAL MANUSCRIPT 640

# THE LIVING CELL
# AS AN OPEN THERMODYNAMIC SYSTEM:
# BACTERIA AND IRREVERSIBLE THERMODYNAMICS

W. Burtis Mercer
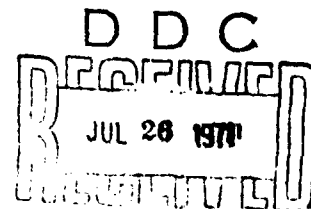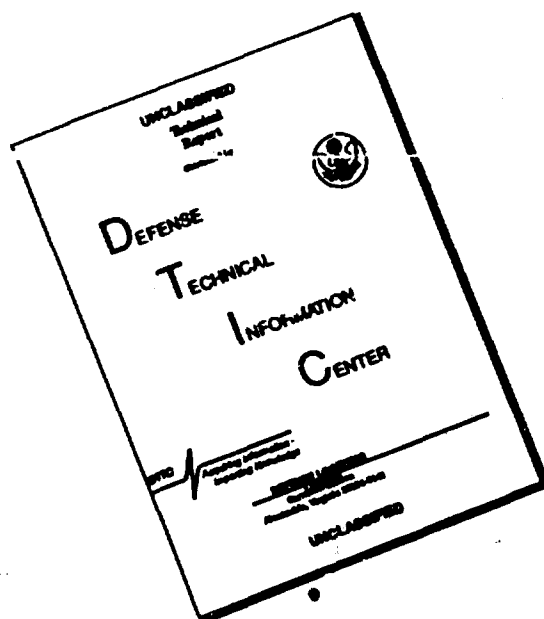
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is available. An hypothesis is presented, considering the living cell
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of the bacterial life cycle. It provides reasons for certain observed
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It provides reasons for certain observed anomalies, showing them
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investigational techniques.

CONTENTS

## I. INTRODUCTION

Bacteria in their natural habitat have an environment whose chemical and physical nature changes but little with time. Living higher animals, in whose organs or tissues various bacterial species are found, are generally endowed with a homeostatic capability. In warm-blooded species, internal thermal regulation is part of the homeostatic condition. Only when bacteria are physically confined and their numbers increase greatly are they faced with a changing environment.

More broadly speaking, bacterial species live and multiply in temperatures ranging from below 0 C[1] to 92 C.[2] Some are found only in fresh water, others only in brines.[3] Some species manage in a wide variety of environments.[1] In all of these situations, the environment does not necessarily change its chemical or physical characteristics with time.

The purpose of this paper is to present a theoretical basis for interpreting all these observations on a single set of premises. A bacterium is defined thermodynamically as an open system. The theory of nonequilibrium thermodynamics, which has been shown to describe accurately other open systems[4] should also apply to bacteria. Indeed, this theory has been successfully applied to other biological systems.[5] After defining the bacterial system and emphasizing the proper laboratory conditions for conducting its study, the application on nonequilibrium thermodynamics to this system will be examined. Some hypotheses based on this application will be presented that provide logical descriptions of bacterial characteristics that are not otherwise understood.

## II. THE BACTERIAL CELL AS A THERMODYNAMIC SYSTEM

### A. DEFINITIONS OF THE SYSTEM AND THE ENVIRONMENT

The individual bacterial cell bounded by the outer surface of the cell wall is the thermodynamic system whose properties and behavior will be discussed in this paper. This definition is adopted in full recognition of certain practical limitations on observation.

A living bacterium grows. At some point during its growth each divides into two bacteria. The "size" of the single cell (by any criterion) at the beginning of division varies.[6,7] So also does the relative "size" of the two daughter cells, but there have been observed lower and upper bounds on cell volume.[8] Bacterial life is, then, a cyclic process, "beginning" with the newly separated daughter cell and "beginning" again when the next generation proceeds to grow as a free entity. Given the proper environment there is no end to bacterial life.

The theory we will apply to the system of interest describes such cyclic processes. The distinction between the single cell as a thermodynamic system and the sum of all cells in a culture - the cells alone - as a thermodynamic system cannot in practice be made, for we are forced to observe a multitude of single cells in order to see anything at all.

The system, considered as a single cell or as the sum of all cells in a culture, is an open one in the thermodynamic sense. Energy, entropy, and matter flow into the system from the environment. Chemical and physical transformations occur within the system, accompanied by the net production of entropy. Energy, entropy, and matter flow from the system to the environment.

The environment of the system includes the suspending aqueous solution of cell nutrients and substances discarded by the cells. The gaseous atmosphere above the suspending solution, the vessel that contains it, and the remainder of the world outside the bounding surface of the system are also parts of the environment.

## B. DESCRIPTION OF THE SYSTEM AND THE ENVIRONMENT

### 1. Physical Features

Extensive chemical and physical investigations have been carried out on this thermodynamic system and on its environment. From these investigations has emerged the hypothesis that the system is composed of three major physical components. The outermost part is the cell wall, which through its mechanical strength delimits the volume of the cell. Just beneath the cell wall lies a very thin membrane, one of whose major functions appears to be that of controlling flow of molecules into and out of the cytoplasm. The cytoplasm is the aqueous solution of many kinds of molecules that fills the cell interior. Organelles are suspended in this solution; how many kinds of organelles per cell, and their function, is still poorly understood.[9]*

### 2. Chemical Features

The bacterial cell wall, cytoplasmic membrane, and cytoplasm contain many substances not initially present in the external environment of whole, growing cells. Many enzymes found in fragments of cytoplasmic membranes are not directly accessible to the environment.[9]** The concentrations in the cell's environment of some molecular species decrease steadily as time passes. Clearly, it is necessary that relatively simple nutrient molecules enter into a pre-existing bacterial cell in order that chemical reactions occur there to produce additional bacterial substance.

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* Pages 216-225.
** Page 386.

Within the system, nutrient substances undergo reactions that produce macromolecular cell components, resulting in an increase in bacterial cell mass (and volume). Energy for the required syntheses is derived also from reactions of nutrient molecules; since chemical reactions seldom have unit efficiency, some energy is lost as heat. Chemical reactions that produce complex molecules from relatively simple ones are generally interpreted as occurring in a coordinated sequence of reactions, each of which produces at least one molecule more complex than those utilized. There is a net reduction in entropy of the matter remaining in the system, and entropy produced in the cells flows to the environment. The final product of a sequence of reactions may be known where the individual steps in the sequence are not.

As the thermodynamic system (the bacterial cell) continues to function, it has been observed that some low-molecular-weight species not initially present in the environment appear there, and their concentrations increase with the passage of time. These same substances, if they are found within the system, appear there only in low, fixed concentrations.[9]* For example, $CO_2$ produced by metabolism of carbohydrates does not accumulate within the system but is released to the environment. It is clear that the system rejects some molecular species produced as by-products of its functioning.

Our knowledge of the system's composition and properties is less clear-cut than our knowledge of the environment. This is not to deny that considerable data have been obtained on the <u>average</u> chemical composition and physical properties of <u>masses of bacteria</u>. A bacterial mass composed of many individual cells must contain many cells in every physiological state between two cell divisions, as well as in process of division. For this reason the data available are averages over the cell growth and division cycle. The molecular chemistry of a single bacterial cell cannot be analyzed with existing techniques; it is unlikely that this situation will change. However, some physical properties, such as net surface charge, can be studied with single cells.

If one had cultures in which growth of all cells were perfectly synchronized with regard to the physiological state of the individuals, the cells could be analyzed chemically at any chosen physiological state. Employing a mass of synchronously growing cells would be, in effect, amplifying the response of a single cell by a determinable factor, the number of cells per unit volume. Kubitschek has reported data from synchronous bacterial cultures.[10] There have been recent claims that such synchronous continuous cultures of the yeast, <u>Candida utilis</u>, can be produced.[11]

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* Page 682.

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That one must, in practice, work with average values of pertinent chemical and physical properties of a system does not prevent making useful thermodynamic studies of that system. Often, kinetic and thermodynamic studies made together provide far more information than either approach made separately.

Dean and Hinshelwood,[12]* as well as many others, have given careful consideration to the chemical species present in bacteria, the reactions that produce them, and the chemical kinetics of the observed reactions. These observations have led to proposals of reaction sequences beginning with nutrient molecules and ending in the formation of bacterial substance and "waste" molecules. Numerous reaction sequences are considered to exist. Many molecular entities appear as members of more than one sequence, thus providing coupling of sequences. The bacterium utilizes whichever overall reaction pathway is necessary to metabolize the nutrients available to it. Dean and Hinshelwood[12]** develop these ideas fully as their Network Theorem and Total Integration principle. The sequence of physical and chemical events comprising bacterial growth, derived from kinetic studies, meets the thermodynamic criterion that the Gibbs Free Energy of the system decreases.

The characteristics of bacteria and their mode of living outlined above have been determined with batch cultures grown at the optimum temperature for the species employed by the experimenter. There are disadvantages to the use of batch cultures. The system is being forced to live in a continually changing environment. The supply and the environmental concentration of nutrient molecules (except for oxygen) diminish rapidly as the system enlarges by cell division, while the environmental concentration of waste molecules rises with proportionate rapidity. Considering the situation from the viewpoint of thermodynamics, the system is neither isolated nor closed, but open. The effect of these rapid, drastic environmental changes requires constant changes in the system's character in order to maintain life. Ultimately, cell replication ceases, and shortly thereafter, for reasons almost totally unexplored, disintegration of the system begins.[9]***

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* Chapter IV.
** Chapter V.
*** Page 371.

## III. NECESSARY EXPERIMENTAL CONDITIONS
## FOR STUDYING BACTERIA AS OPEN SYSTEMS

### A. CONTINUOUS CULTURE

It is here emphasized that the only proper laboratory environment in which one should study bacterial growth and function is one that is time-invariant. Continuous culture of bacteria in such systems as the "chemostat"[13] or the "turbidistat"[14] is capable of providing such an environment. The literature reports several attempts to study bacteria under environmental conditions more nearly time-independent than the usual batch culture. Most of these only approximate the desired environmental condition, each falling short in one way or another. For example, periodic replenishment of a nutrient[15] is not identical to maintaining time-invariant the concentration of that substance.

Use of chemically defined media is strongly recommended to obtain accurate and continuous control of nutrient chemical species in the environment and to make less difficult the identification and quantitation of molecules discarded by the system. It is desirable to design continuous culture systems in which pressure, temperature, gaseous atmosphere, and illumination are also subject to accurate and precise control, for these are important externally applied constraints on the system.

### B. STEADY STATE OF THE SYSTEM

As bacteria multiply in a continuous culture apparatus, the population density of the cells becomes established, in a reasonably short time, at a fixed level. New cells replace those that leave with the effluent because the thermodynamic system (bacterium) has attained a "steady state" of growth and replication.

## IV. THE STEADY STATE CONCEPT

Difficulty arises from interchangeable use of the terms "equilibrium" and "steady state", thus tacitly ascribing to these terms an equivalence that, from a physical-chemical standpoint, they do not have.

In any steady state there are no changes with time of intensive thermodynamic variables of the system. Thermodynamic potential differences between system and environment are maintained at some non-zero value because of constraints applied through the environment to the system. Examples are chemical potential difference of a solute, pressure gradient, and temperature gradient.

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Time-invariance of the constraints is necessary if the system is to achieve a steady state. After constraints are applied, thermodynamic parameters of the system change with time until they attain magnitudes that are mutually compatible and that are compatible with the externally applied constraints. Thereafter they do not change with time.

Altering the magnitude of an external constraint after the system reaches the steady state results in changes in the thermodynamic parameters of the system until a new steady state is reached.

Equilibrium is a condition approached or attained by a thermodynamic system (bacterial or other), given sufficient time (and it may be a very long time) when there are imposed on the system no time-invariant external constraints.

Were one to come upon a system whose thermodynamic properties were observed to be unchanging in time, how could one decide whether the system were in a steady state or at equilibrium? The question can be resolved by isolating the system, that is, by putting the system in a situation where it can exchange neither matter nor energy with its environment. A system in a steady state would undergo changes for a while after it was so isolated; a system at equilibrium would not change at all. Recall the statement concerning flows through open systems in Section II, A. Steady state cannot exist in isolated systems.

In order to sharpen the distinction between equilibrium and steady state let us consider an example. An open thermodynamic system, which in many ways is closely related to living systems, can be observed in a candle flame. The thermodynamic system proper is that region of chemical reaction that is the flame. Heat leaves this system (energy exchange with environment) and is (in part) utilized to melt and vaporize wax, which then enters the system (matter exchange with the environment). Oxygen diffuses into the flame from surrounding gases; carbon dioxide and water vapor leave the flame (further matter exchange with environment).

The flame responds to changes in external constraints. If the flame's oxygen supply is reduced by diluting the surrounding air with nitrogen, it is possible to reduce the rate of chemical reaction in the flame.

Before the point of flame destruction by oxygen starvation is reached, limitation of oxygen supply will reduce flame size and rate of heat production. If rate of heat production is sufficiently curtailed in this manner, wax will not be melted and vaporized fast enough to keep the system supplied with fuel and the flame will cease to exist. The flows of fuel, oxygen, reaction products, and heat are interdependent.

There is no such thing as a thermodynamically isolated or a thermodynamically closed flame; each such system is an open system. It operates under a set of externally applied constraints, and only when those constraints individually and collectively lie within certain limits. As

long as the constraints are unchanged the system is in the appropriate
steady state. The characteristics of the system change as each constraint
is changed, but this system has no point of equilibrium.


## V.  NONEQUILIBRIUM THERMODYNAMICS IN MICROBIOLOGY:
## LINEAR DESCRIPTIONS


### A.  CRITERION OF SPONTANEOUS CHANGE

The utility of a decrease in Gibbs Free Energy as the criterion for
occurrence of a spontaneous change is limited to events occurring under
conditions of constant temperature and pressure. It was pointed out in
Section III, A, that understanding, and hence controlling, bacterial
viability will require studying cell responses when pressure and temperature
are among the elective external constraints on bacterial growth. Therefore
a more general criterion of spontaneous change is needed.

The only true general criterion for spontaneity of change in any
system is the behavior of the global entropy accompanying that change.
This quantity always increases in any spontaneous (irreversible) change.
Entropy change comes about in two ways:  (i) entropy flow between system
and environment, or (ii) production of entropy within a system. Non-
equilibrium thermodynamics (the thermodynamics of irreversible processes)
concerns itself with the quantitative expression of entropy production
within a system.

Entropy is a defined quantity, not subject to direct experimental
evaluation. It is therefore necessary to express entropy production in
terms of experimentally measurable quantities. The form taken by the
entropy production per unit time per unit volume is a sum of terms, each
of which is defined as the product of a flow and the conjugate force that
drives the flow. For example, the entropy production accompanying a flow
cf heat has a temperature function as the conjugate force. Similarly,
affinity is the driving force for a chemical reaction. The basic ideas of
nonequilibrium thermodynamics are outlined in Section VII.

A vast quantity of experimental evidence supports the commonly accepted
view that simple free-living microorganisms have available to them
numerous metabolic pathways. Utilization of these pathways by the micro-
organism implies flows of matter and heat into and out of the organism,
flows that are accompanied by entropy production. It also implies a
variety of chemical reactions within the organism, accompanied by further
production of entropy. In order to apply nonequilibrium thermodynamics
to the study of these organisms successfully, it will be necessary to begin
by assuming the simplest possible sequence of metabolic reactions of all
those that are believed capable of occurring in the cell species.

## B. LINEAR LAWS

We are required to write the linear laws relating flows and forces, whose product is, in sum, the entropy production of the total process of bacteriological life. In many other open (non-biological) systems the conjugate forces and flows are easily determined by inspection of the system. Having done this we are in a position to write the linear phenomenological equations. The set of phenomenological equations that describe bacterial growth must of course include, in addition to equations for chemical reactions within the cell, equations describing the entry of nutrient materials and the exit of waste materials as well as those for energy exchanges with the environment.

The set of equations can be tested for validity by experimentally determining the necessary phenomenological coefficients to obtain a self-consistent set that describes the behavior of the bacterium. It is important to note that there may be more than one way to define the flows and forces that can be used for a mathematical description of any given system. In order to obtain useful information about the system, we must be careful to choose forces and flows that are conjugate and can be measured experimentally.

Consider, for example, that it has been determined that the functioning of some cells maintained in a time-invariant nutritional environment can be described by this set of linear laws:

$$J_1 = L_{11}X_1 + L_{12}X_2 + L_{13}X_3 + L_{14}X_4$$

$$J_2 = L_{21}X_1 + L_{22}X_2 + L_{23}X_3 + L_{24}X_4$$

$$J_3 = L_{31}X_1 + L_{32}X_2 + L_{33}X_3 + L_{34}X_4 \tag{1}$$

$$J_4 = L_{41}X_1 + L_{42}X_2 + L_{43}X_3 + L_{44}X_4$$

where

$J_i$ = flow of heat, chemical substance (by diffusion), or rate of a chemical reaction ($i = 1,2,...,n$),

$X_j$ = force conjugate to flow $J_i$; temperature function, chemical potential difference, or affinity. ($j = 1,2,...,n$).

$L_{ij}$ = numerical constants. Called "straight coefficient" if $i = j$, "cross coefficient" if $i \neq j$. $L_{ij} = L_{ji}$ if $i \neq j$.

Equation (1) indicates that one must determine experimentally and simultaneously each of the flows $J_i$ occurring when each force $X_j$ has a known value in order to calculate the values of the constants $L_{ij}$. The experimental problem is reduced because of the Onsager Reciprocal Relation. This sort of study would require use of a continuous-flow

culture apparatus such as that originally described by Novick and Szilard[13] in order to fulfill the condition that the nutritional environment be time-invariant.

When one deals with bacteria it would be most difficult to determine directly the chemical potential of any substance, even sodium ions, inside a bacterial cell. Often such difficulties can be circumvented by employing equations reciprocal to equation (1), which are:

$$X_1 = R_{11}J_1 + R_{12}J_2 + R_{13}J_3 + R_{14}J_4$$

$$X_2 = R_{21}J_1 + R_{22}J_2 + R_{23}J_3 + R_{24}J_4$$

$$X_3 = R_{31}J_1 + R_{32}J_2 + R_{33}J_3 + R_{34}J_4 \tag{2}$$

$$X_4 = R_{41}J_1 + R_{42}J_2 + R_{43}J_3 + R_{44}J_4$$

where

$$R_{ij} = \frac{|L|_{ij}}{|L|}, \quad |L|_{ij}$$ being the minor of the determinant corresponding to the coefficient $L_{ij}$ and $|L|$ the determinant of the matrix of all the coefficients $L_{ij}$.
For example,

$$R_{11} = \frac{\begin{vmatrix} L_{22} & L_{23} & L_{24} \\ L_{32} & L_{33} & L_{34} \\ L_{42} & L_{43} & L_{44} \end{vmatrix}}{\begin{vmatrix} L_{11} & L_{12} & L_{13} & L_{14} \\ L_{21} & L_{22} & L_{23} & L_{24} \\ L_{31} & L_{32} & L_{33} & L_{34} \\ L_{41} & L_{42} & L_{43} & L_{44} \end{vmatrix}}.$$

When equation (2) is used to relate flows and forces, the phenomenological coefficients are generalized resistances rather than generalized conductances. The Onsager Reciprocal Relations still apply to the $R_{ij}(i \neq j)$.

Equation (2) gives the forces as functions of the flows. The _fluxes_ of particular chemical species into or out of the cell can frequently be determined with considerable accuracy by use of the analytical technique currently available. (It is for this reason that the use of chemolithotrophic autotrophic bacteria to test the hypothesis presented here is suggested. Organic molecules present must certainly have been produced by the cells. Bacteria capable of growth in chemically defined media

with glucose or glycerol as carbon source should also be useful.) Even the small quantity of heat flowing from bacteria can be measured with considerable precision by differential calorimeters. The great value of having both sets of equations (1) and (2) arises from the fact that by proper manipulation of the experimental situation, one or more of the flows or forces can sometimes be held to the value zero. This leads to simpler relationships among the constants and the experimental quantities, and it is easier to evaluate specific constants.

Should the temperature of the environment (and hence of the cell) be set at some new value in a chosen sequence, it may well be determined that one or more specific numerical constants, $L_{ij}$, take on new values for each new temperature. With these changes, the same forces and flows may serve to describe the functioning of the bacteria. On the other hand it might be observed, for example, that whereas the equations given above describe the behavior of a thermophilic bacterium in its natural environment, this same organism may be able to survive and grow at a much lower temperature than that of its natural environment but that the above equations do not describe the bacterial functions at all at the lower temperature. Experiment would be required to find a proper set of linear equations similar in form to those above. Describing bacterial functions under these conditions might require equations for, say, six flows, each equation having six terms. In any case, some of the cross-coefficients may be zero, or some may be negative.

Were one to examine the steady states sustained by bacterial growth in continuous culture as affected by the concentration of nutrient in the environment to determine the phenomenological coefficients for a single species of organism with several alternative carbon sources, taken singly, one would be able to compare the efficiency of utilization of those carbon sources by the organism under the specified conditions. Alternatively, one could adhere to a single carbon source and observe the phenomenological coefficients for several organisms under fixed environmental conditions, thus comparing the efficiency of organisms in the utilization of a given source of carbon. Changes in numerical values of the phenomenological coefficients would signal alterations of relative importance of metabolic reactions; failure of the initial set of phenomenological equations to describe the system with any set of constant coefficients would be proof that the initially applicable metabolic pathways were no longer being followed.

A means of measuring efficiency of energy conversion has been developed for conditions in which the Linear Laws hold.[16-18] They describe coupling in multiple flow systems and express the efficiency of energy conversion in such systems. Extent of coupling and efficiency are expressed in terms of the phenomenological coefficients.

## C. FORCES AND FLOWS IN LIVING SYSTEMS

### 1. Temperature Difference and Heat Flow

There is a temperature difference between the interior of a living bacterial cell and the nutrient medium that surrounds the cell. This is a thermodynamic necessity.

In nature nothing moves unless it is driven. Sensible heat moves from one point in space to another point in space only because there is between those two points a temperature difference that drives the heat flow. Nutrient medium that suspends growing bacteria becomes heated.[19-21] There must therefore be a temperature difference between the interior of the bacteria and the environment in which an increasing quantity of heat is detected. Calculations by the author based on data from Forrest and Walker[19] indicate a temperature gradient for Streptococcus faecalis of about 0.4 degree per cm. Although this difference is small, it is the driving force of a non-negligible heat flow and the product of the force and heat flow contribute to entropy production.

We hypothesize that each species of bacterium or other micro-organism will tolerate a growth temperature range controlled by the rate and manner of entropy production. Part of the entropy produced will appear outside the cell as sensible heat. The fraction of all entropy produced that appears in this form depends on the nature and number of entropy-producing processes occurring in the cell. Temperature difference between environment and organism interior as well as the internal temperature of the cell is undoubtedly affected as the environmental temperature is altered. Rate of heat flux from within the cell will be affected by temperature drop across cell boundaries in accordance with Newton's Law and the heat conductivity of the cell and its boundary layers. Consequently a "feedback" effect is to be expected and a steady state of internal cell temperature will be attained. The internal temperature at the steady state characteristic of a bacterial species will govern chemical reaction rates within the cell and metabolic diffusion rates within the cell and through the cell boundary.

Consideration of the temperature difference between environment and cell interior opens the way to a reasonable explanation in terms of non-equilibrium thermodynamics for the observations of bacteria able to live at temperatures that inactivate most enzymes in vitro. Brock[2] states that there is no evidence that organisms die because of heat inactivation of proteins or other macromolecules. He suggests that the stability of thermophilic organisms can be attributed to membrane structure properties of these organisms, which in some unknown manner differ from those of mesophiles or psychrophiles. In terms of the hypothesis set forth here, thermophilic organisms are stable at their observed temperature because at that environmental temperature the thermodynamic force related to temperature difference between environment and cell interior is compatible

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with the other thermodynamic forces (affinity, chemical potential difference function) existing in that system. All these forces will bear on those metabolic processes that, cumulatively, affect cell membrane integrity. The temperature gradient contribution to the flow of substances through the cell membranes of bacteria and thus its influence on metabolic processes probably differs widely as membranes differ widely. In some cases this contribution may assume an importance it apparently does not have in others.

There is nothing in this hypothesis to preclude alterations in mechanisms or rates of entropy production by genetic changes[22] or by adaptations of the sort described in detail by Dean and Hinshelwood.[12]*

2. Chemical Potential Difference and Mass Transfer: Activated Transport

Another combination of flows and forces contributing to entropy production by microorganisms is that of mass flow and difference in chemical potential. Nutrients and metabolic products pass through the exterior layers of the cell at a rate that depends on the nature of the cell wall, the specific substances, and their chemical potential differences across the exterior layers. Whether the passage of a particular substance is purely by chemical diffusion or whether it requires the expenditure of metabolic energy to operate an enzyme system has been debated at great length over a number of years.[23] In view of the cross-linking of the various flows involved in the operation of thermodynamically open systems, one must admit the possibility that those molecules that appear to pass by diffusion driven solely by a difference of chemical potential may in fact be driven by a combination of forces.

The magnitude of flow of any one substance caused by the non-conjugate forces may be quite small or it may be quite large; furthermore, the flow of a substance driven by non-conjugate forces may be in the same direction as that due to the conjugate force or it may be in the opposite direction. Magnitude and direction of these cross-effects must be determined experimentally.

Note that the force driving diffusion is stated to be the <u>chemical potential difference</u> of the substance across the exterior layers of the cell, and not the <u>concentration difference</u>. Although these quantities are related, they are not the same. It is essential to deal with the proper forces and flows when combining these quantities to determine entropy production.[24]

D. APPLICABILITY OF LINEAR LAWS

One cannot always state a priori that the Linear Laws will hold for a specific open system. A certain amount of preliminary experimentation

* Chapters VI-X.

may be necessary. Forrest and Walker[25] have questioned the applicability
of the Linear Laws to living bacterial cultures. Their work was done on
batch cultures of S. faecalis. However, in a recent publication,
Kubitschek,[10] working with synchronous cultures (which is equivalent to
amplifying a single cell), has demonstrated cell volume increase with time
of Escherichia coli that is linear through two generations. This work also
suffers the disadvantage (from the present viewpoint) of having been done
in batch cultures, but it does have the mitigating circumstance that the
growth used up only a very small portion of the available nutrients and
produced very small amounts of any waste products, and therefore, to some
degree, circumvented the limitations of batch culture technique.

Biological membranes, some complex, have already been examined in the
light of the theory of irreversible thermodynamics. Hempling[5] employed
this formalism to describe function of ascites tumor cell membranes;
Katchalsky and Curran[26]* applied the method to transport through toad skin,
using data from the literature.

It is often considerably easier to work with synthetic membranes,
partly because they can be made to have a larger area than is available
from even the larger single-celled species of microorganisms, and partly to
avoid the complications of a complex metabolizing membrane derived from
multicellular organisms. Synthetic membranes have been shown capable of
demonstrating at least some of the properties of living membranes by
Shashoua,[27] who used a polyelectrolyte synthetic membrane to study current-
voltage relationships. He was able to demonstrate the ability of this
membrane to "fire" in the manner of a neuronal membrane. Blumenthal,
Caplan and Kedem[28] have used linear equations with constant phenomenological
coefficients to describe coupling of an enzymatic reaction to transmembrane
flow of electric current in a synthetic "active transport" system involving
enzymes.

Hempling[5] demonstrated the usefulness of an analog computer in working
with the phenomenological equations.

## VI.   NONEQUILIBRIUM THERMODYNAMICS IN MICROBIOLOGY:
### NON-LINEAR DESCRIPTIONS

### A.  POSSIBLE BREAKDOWN OF LINEARITY

In the preceding part of this paper, it was pointed out that the Linear
Laws have been found useful in describing some real systems. The use of
linear kinetic equations is known to yield agreement with experiment in
cases near equilibrium. Under such conditions, affinities of the chemical

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* Page 123.

reactions occurring are small. In none of the previously discussed experimental work has a direct attempt been made to ascertain whether the affinities are indeed small; it has been implied that ability to describe the system with Linear Laws assures this to be true. The appearance, with time passage, of new phase boundaries within the real system has been ignored in using linear equations derived from nonequilibrium thermodynamics.

If a system exhibits steady states both near equilibrium and far from equilibrium, there are several questions of interest: (i) Is the system far from equilibrium then still composed of the same phases as before, or have changes occurred? (It is clear that if a bacterium is to grow and divide into two cells, it must be possible for the homogeneous cytoplasm to give rise to cell walls - i.e., non-homogeneity must appear.) (ii) Has there occurred any change in the condition of _spatial_ stability as the system is removed further from equilibrium? (A phase is defined as a volume of matter whose properties are uniform throughout. A system is spatially unstable if it is capable of simultaneously existing in more than one phase.) (iii) What is then the condition of _temporal_ stability - is a small perturbation of some thermodynamic property followed by return to the steady state? and (iv) Do the Linear Laws that describe systems near equilibrium still apply?

## B. SYSTEMS INVOLVING LARGE AFFINITIES

Prigogine and his co-workers have investigated conditions of spatial stability of open systems and the possibility that homogeneous systems that have steady states near equilibrium may also have steady states far from equilibrium. They have shown that in some systems there are steady states both near equilibrium and far from equilibrium. In these cases the same chemical reactions account for events under both sets of circumstances. Prigogine and Nicolis[29] have carried out an investigation on a biological model proposed by Turing;[30] two simpler models were investigated by Prigogine and Lefever.[31]

The investigation of Turing's model by Prigogine and Nicolis will be summarized. This model appears to represent in broad outline the system that is the bacterial cell.

In Turing's model two initial substances, A and B, are transformed into two final products, D and E, through the intermediate products, X and Y, by the action of the catalysts C, W, V, and V' following the general scheme:

$$A \longrightarrow X \longrightarrow C \longrightarrow D$$
$$\text{(C,W)} \qquad \text{(V,V')}$$
$$B \longrightarrow Y \longrightarrow E \qquad\qquad (3)$$
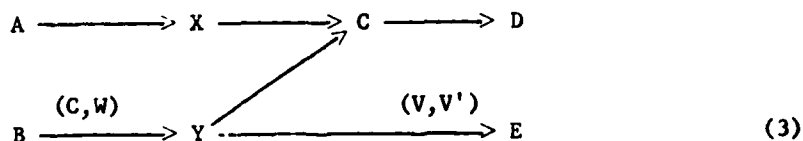
Turing has proposed the following set of chemical reactions to occur:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X$$

$$X + Y \underset{k_3}{\overset{k_2}{\rightleftharpoons}} C$$

$$C \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} D$$

$$B + C \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} W \qquad (4)$$

$$W \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} Y + C$$

$$Y \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} E$$

$$Y + V \underset{k_{-8}}{\overset{k_o}{\rightleftharpoons}} V'$$

$$V' \underset{k_{-9}}{\overset{k_9}{\rightleftharpoons}} E + V$$
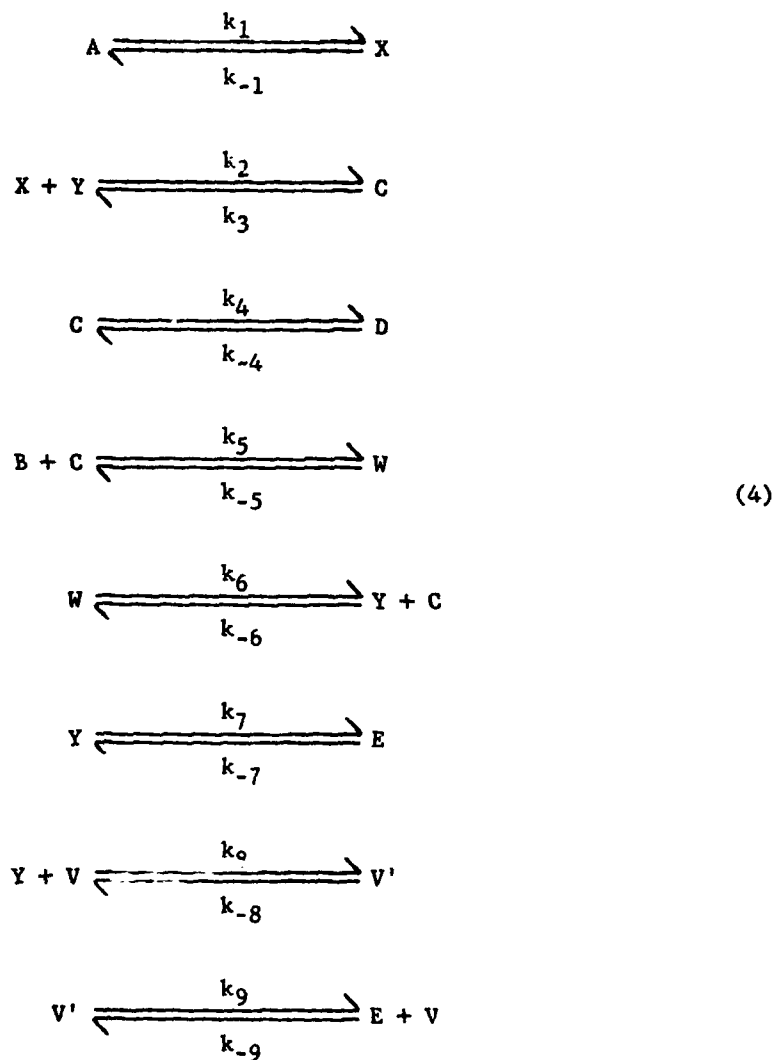
These equations may be summarized:

$$A + B \underset{}{\overset{k_1 k_2 k_4 k_5 k_6}{\rightleftharpoons}} D \qquad\qquad (5a)$$

$$B \underset{}{\overset{k_5 k_6 k_8 k_9}{\rightleftharpoons}} E \qquad\qquad (5b)$$
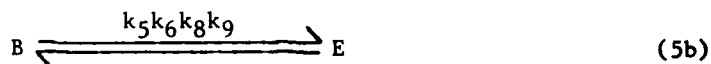
Prigogine and Nicolis[29] show that Turing's model is stable in a steady state near equilibrium. In this situation the affinities of reactions [equation (5)] are small, and the requirements of linear irreversible thermodynamics are met.

Consider equation (3). The carbon source (analogous to B) serves as precursor for bacterial mass (D) and for discarded molecules (E) from which energy is extracted for combining A and B and for all other reactions requiring energy. The reactant A represents the nitrogen source and the other substances required for cell function. In order for the system to function, certain catalysts, i.e. enzymes (C, W, V, V'), must be present as one produces new bacterial mass only from pre-existing living bacterial mass. The reaction $k_{-7}$ in equation (4) may be construed to provide for feed-back control on rate of energy availability.

In their analysis Prigogine and Nicolis[29] investigate the stability in space and in time of the system when affinities of reaction in equation (5) are large, and when one takes into account diffusion processes in the system. Under these conditions it develops that when (i) the external constraints are time-invariant (i.e., concentrations of A, B, D, and E are constant) and (ii) the affinity of equation (5a) is very large, the system becomes spatially unstable. One or more new phases can appear. Their analysis also shows that the appearance of inhomogeneity depends on the efficiency of the enzyme V'. They have shown that it appears likely that there is a critical range for the affinity value. Beyond a transition point in this range the stable steady state is inhomogeneous and two or more phases coexist.

Prigogine and Lefever[31] have also considered the question of temporal stability of systems such as that proposed by Turing,[30] or of a similar one observed by Chance et al.[32] Prigogine and Lefever have shown that <u>oscillation around an unstable steady state</u> is a typical phenomenon of systems far from equilibrium.

The present author views bacterial cell growth and division, taken together, as a single process that is amenable to interpretation as a process of oscillation about an unstable steady state. We shall next summarize the characteristics of such oscillatory systems and present an hypothesis that interprets the bacterial cell life cycle as such a system.

## C. OSCILLATION (OR CYCLING) ABOUT A STEADY STATE

Glansdorff and Prigogine[33] and Prigogine and Balescu[34,36] demonstrated that cyclic processes in open systems with constant external constraints can be understood in terms of the theory of nonequilibrium thermodynamics. If one observes the affinities of various chemical processes occurring within an open system, the affinities, considered to be distances on a graph of two or three dimensions, may be plotted against each other. The number of axes required depends on the number of independent affinities required to describe the processes occurring. On the plot, the overall affinity of the process is represented by the linear distance from the origin to the plotted point. We deal here with directed magnitudes; the affinities are vectorial quantities. Of course, if more than three independent affinities are required, one can depict graphically only selected affinities. The state of the system under study is characterized by a point in "affinity space", the terminus of the overall affinity vector.

In terms of such plots in affinity space, Glansdorff, Prigogine and Balescu[33-35] have reached these conclusions:

1) The findings are in accord with the second law of thermodynamics: that is, the origin of the plot, where all affinities are zero, is the thermodynamically stable equilibrium state. (If a stable system is removed a small distance from that state by any means it will return spontaneously to the stable state.)

2) Nonequilibrium stationary states may exist for a given system. Such a state is characterized by non-zero values of some or all of the independent affinities definable for the system. The affinities must be positive quantities.

3) Near equilibrium, i.e., for small affinities, nonequilibrium stationary states are always stable.

4) In cases where the phenomena involve large affinities, i.e., are far from equilibrium, nonequilibrium stationary states may be stable or unstable. On a plot in affinity space a fixed point characterizes the stable stationary state. Characteristics of unstable stationary states depend on the number of independent irreversible phenomena occurring:

a) Even number of independent irreversible phenomena: For a given set of external constraints one, and only one, unstable stationary state exists. If the system is perturbed by any means, the system will be unable to return to the unstable stationary state. The point in affinity space that represents the state of the system at each instant will move in a closed path around the point that characterizes the unstable nonequilibrium stationary state. The sense of rotation is fixed by the rate at which entropy production varies with respect to the affinity changes.

b) Odd number of independent irreversible phenomena: There exists an infinity of possible nonequilibrium stationary states for a given set of external constraints.

The fact that a single, nonequilibrium, unstable stationary state arises from operation of an _even_ _number_ of independent irreversible phenomena is not at all intuitively obvious. Mathematical analysis such as that of Glansdorff, Prigogine. and Balescu[33-35] is required to reveal this fact.

## D. SINGLE-CELL FUNCTION IN NONEQUILIBRIUM THERMODYNAMIC TERMS: AN HYPOTHESIS

Taking as initial justification the analysis of realistic reaction models for living systems outlined in Section VI, B, it is claimed that nonequilibrium thermodynamics provides a reasonable basis for understanding the operation of the living cell.

The chemical reactions that occur in cell growth are known to be not at equilibrium by the very nature of the process. In fact, the appearance of nonhomogeneities, i.e., production of cell membranes, of cell walls, as a result of these processes indicates that these phenomena involve non-equilibrium stationary states far from equilibrium.

In different growth media chemical and physical properties of bacteria within a given strain of one species show marked differences.[9]* This has also been shown for a yeast, _Candida_ _utilis_.[36] Growth temperature has been shown to be a significant variable in continuous culture of a yeast, _Saccharomyces_ _cerevisiae_ IGC 3507,[37] and of bacteria, _E._ _coli_ B.[38] Observations reported on _S._ _cerevisiae_ LBGH 1022[39] and on a bacterium, _Aerobacter_ _cloacae_,[40] demonstrate that for a given set of time-independent external constraints there results (in continuous culture) a single characteristic cell population density. All this taken together implies a causal relationship between external constraints and the stationary state of living bacteria and yeasts. It indicates that a _single_ non-equilibrium stationary state exists for each set of independent external constraints.

The cited evidence leads us to the hypothesis that there is, for each set of independent time-invariant external constraints imposed on a cell (or cell culture), a single unstable nonequilibrium stationary state of the system. Consequently there is an _even_ _number_ of independent irreversible phenomena that characterizes cell life functions. (The reports noted discuss yeasts and bacteria. As will be indicated later, it seems reasonable that this hypothesis may also apply to mammalian cells.)

* Page 364.

The number of independent affinities characterizing cellular growth is probably more than two. It is a matter of repeated experimental observation that in growth processes in chemically defined medium, part of the carbon source (often glucose) is converted to carbon dioxide or other "waste" substances and part of it into cell substances whose molecules are much more complex than glucose. A similar statement may be made concerning the nitrogen source. Other chemical substances - phosphates, metallic ions - are necessary for cellular function. External pH, temperature, and solute concentration affect cell function. Hence it seems reasonable to conclude that the minimum number of independent irreversible phenomena characteristic of cell growth and division is four. It may well be larger, but it is an even number.

To apply the rationale of nonequilibrium thermodynamics to the life cycle of cells, it is necessary to recognize that nonlinear flow equations (that is to say, nonlinear rate equations) must be used to describe the system, and such descriptions require recognition of some specific set of kinetic equations. When we deal with systems near equilibrium, no specific mechanism for the kinetics of the process is required. It is only when one must deal with systems far from equilibrium, systems involving processes with large affinities, that one must take into account specific mechanisms.[41]*

The affinity of essential reactions associated with bacterial growth and division must have at least a minimum value. This idea has experimental support.[9]** It is hypothesized here that the minimum value requirement applies to the individual independent affinities and not alone to the resultant overall affinity.

In the process of cell growth and division the point (in affinity space) representing the system at any instant moves in a closed curve around the point characterizing the single unstable stationary state. One complete "rotation" of the system through the closed curve traced out in affinity space by the point characteristic of the system is accompanied by division of the cell. Cycles of division following growth, under time-invariant external constraints, will continue indefinitely.

The growth rate of bacteria at a fixed temperature is a function of both composition of the medium and chemical potentials of the component substances.[9]*** The metabolic pathways that must be followed to utilize the available nutrients thus define the affinity space that applies. The magnitude of affinities is a function of nutrient concentration, of pressure, and of temperature. Chemical potentials of nutrients in the growth medium will influence the time rate of travel along the curve in affinity space of

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* Page 55.
** Pages 365 and 691ff.
*** Page 364.

the point that characterizes the system at any instant. Such control will be limited in the sense that there is a maximum rate at which each germane chemical reaction will occur under the existing constraints; for example, this maximum rate is a function of environmental temperature and of the physical properties of the nonhomogeneous bacterial cell.

It is further hypothesized that under certain quite specific conditions the cell system functions in a stable nonequilibrium stationary state. Under these conditions a bacterium grows and produces entropy at a finite, time-invariant rate. New bacterial mass continues to be produced but, since the point in affinity space that represents the system at any instant does not move as time passes, cell division does not occur. The necessary highly specific conditions for such a change are brought about by forcing the cell to alter its pattern of metabolic pathways. The new pattern is such that the differential equation of entropy production as a function of the forces (affinities) becomes an exact differential (although it still includes an even number - four or greater - of independent irreversible phenomena).

Specific examples of such occurrences may be observed in growth of mammalian cells and in growth of bacterial cells. It has been reported in the literature[42] that addition of tetrazolium compounds to cultures of E. coli B in nutrient broth reduces the mass of bacteria produced per unit volume of medium per unit time. We have noted that, with blue tetrazolium in particular, the bacterial mass produced under these conditions is almost completely (in terms of mass) in the form of filamentous bacteria. Many filamentous cells estimated to be well in excess of 100 microns long have been observed in such cultures. The diameter of these filaments is that of "normal" E. coli. Rosenberg et al.[43] observed filamentous growth of E. coli B in the presence of 1 to 20 $\mu$g/ml concentrations of $(NH_4)_2PtCl_6$. Upon removal of the platinum compound from the bacterial environment, the elongated cells divided into cells of "normal" lengths. Rosenberg et al.[44] recently reported that mammalian cells grown in vitro in the presence of platinum chloride complexes fail to divide.

It is suggested that experiments introducing to bacteria such completely foreign molecules as those mentioned above can aid in exploring further the hypotheses presented here.

## E.  BATCH CULTURES

The theory of nonequilibrium thermodynamics has been developed to its present state under the postulate of time-independent boundary conditions. In terms of practical application to bacterial cell growth, division, and function, this postulate allows quantitative statements only when such conditions prevail. Without further theoretical development this formalism cannot be applied quantitatively to batch cultures, although it can provide some general insights. The following paragraphs present some ideas concerning bacterial growth in batch cultures.

The "lag phase" is the period in batch cultures during which the condition of time-invariant external constraints is most nearly met. The "lag" is considered to end with the first division of the cells of the inoculum. Prior to division the cells of the inoculum have attained an unstable stationary state of growth; departure from the unstable state is initiated by some inevitable perturbation, perhaps of an enzyme function, caused by reduced availability of its substrate molecule whose diffusion rate is altered by phase changes within the cell. At the initial cell division, rotation of affinities about the unstable stationary state has completed the first cycle of the closed curve it would continue to trace out if the external constraints were to remain time-invariant.

The "exponential phase," the "stationary phase," and the "phase of decline" of batch cultures are artifacts of the technique and nothing more. They are reproducible only to the extent that the entire set of circumstances under which they are observed is reproducible.[9]* The exponential phase, it is maintained here, is a steady state of growth only by definition as such for purposes of convenience. The fundamental condition of time-invariant external constraints is not met in batch cultures in "exponential phase," the "stationary phase," or the "phase of decline."

In batch cultures, the reduction in nutrient concentration in extra-cellular environment as a result of cell growth will cause a reduction in affinities of some substances directly, and of others, indirectly. This reduction causes a cessation of growth. In some cases, end-products of metabolism appear outside the cell as carboxylic acid anions, whose apparent toxicity has been considered.[45,46] In batch cultures, the apparent toxicity of metabolic end-products increases with decreasing pH. The uncharged molecular form of any carboxylic acid passes more easily through the cell's negatively charged exterior layers than does the anionic form. (The cell's exterior layers probably undergo some reduction in net charge as the concentration of protons in the surrounding fluid increases. This enhances ability of carboxylates to penetrate the cell, and the apparent toxicity of these entities is consequently increased.) The cell's internal concentration of carboxylic acid in either form will rise as carboxylate re-enters (or fails to depart), leading to lowered affinity of the metabolic reaction producing it. The feedback principle operates, and vital functions cease.

In the final analysis, the toxicity to bacteria of end-products of their metabolism is probably an artifact of the use of batch cultures. If there is an inherent toxicity of end-products in the some sense that such foreign entities as azide ion, cyanide ion, or heavy metal ion are toxic, evidence for this toxicity can be obtained only when all other external constraints are held time-invariant.

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* Pages 363-370.

## VII. THERMODYNAMIC CONCEPTS

### A. ENTROPY CHANGE

Classic thermodynamics limits itself to the statement that the total change in entropy of a closed system ("global" or "universal" entropy change) during a chemical or physical process occurring within that system is either greater than, or equal to, zero. When it is equal to zero any process occurring within the system is reversible. The change in entropy of the closed system is greater than zero for all irreversible processes; all spontaneously occurring processes are irreversible. For closed systems this may be represented simply in the following manner:[47]

$$dS \geq 0 \qquad (6)$$

The expression for entropy change of an open system (such as a single bacterial cell) may be written in two parts as follows:[48,48]

$$dS = d_e S + d_i S \qquad (7)$$

where

$dS$ = total entropy change of the system

$d_e S$ = the part of total entropy change resulting from exchange of energy and matter between the system and the environment; may be positive, zero, or negative with respect to the defined system.

$d_i S$ = entropy change due to physical and/or chemical changes within the defined system; may be zero or positive. This quantity is identical to the $dS$ of equation (6). The terminology is confusing.

By separating the total entropy change of a bacterium into two parts in the manner described it is assumed that entropy is produced within the cell in a closed system. The entire time span of cell life is considered to be the sum of incremental time periods during which incremental changes occur. If the defined system (in the present case, the bacterial cell) be divided into two (or more) subsystems, such as cell cytoplasm (I) and cell covering (II), then the entropy production of the entire system is

$$d_i S = d_i (S^I + S^{II}) \geq 0$$

$$= (d_i S^I + d_i S^{II}) \geq 0.$$

In order not to violate the requirement imposed by equation (6), entropy production by every macroscopic region of the system must separately be greater than or equal to zero:

$$d_i S^I \geq 0 \quad , \quad d_i S^{II} \geq 0.$$

It is forbidden that there be production of entropy in one element of volume followed by consumption of that entropy in another element of volume, however close in space such separate elements of volume may be. Entropy production and absorption must occur within a single volume element. The principle is manifest in metabolizing systems through the coupling of reactions - production by one reaction in a sequence of a molecule required in the next reaction of the sequence. Coenzymes are thus seen to be thermodynamic necessities. Only because coenzymes exist is it possible to carry out chemical reactions in controlled steps, the sites of the steps being well separated in space, as is done in all living systems. Such a formulation is called "local" formulation, as contrasted to the "global" formulation of classical thermodynamics. The term "macroscopic region" refers to any region containing sufficient molecules so that a spontaneous fluctuation in any thermodynamic property is negligibly small in comparison with the average value of that property over the region.

Nonequilibrium thermodynamics is concerned with the quantitative evaluation of $d_i S$ for spontaneous processes. Changes of this nature may be exemplified by some conformational changes of protein, necessitating including changes in extent of hydration. Such reactions are involved in the growth and function of bacteria.

## B. THE GIBBS EQUATION

$$dS = \frac{1}{T} \, dU + \frac{P}{T} \, dV - \sum_\gamma \frac{\mu_\gamma}{T} \, dn_\gamma \tag{8}$$

where

$T$ = thermodynamic temperature, usually on the Kelvin scale.

$dU$ = energy exchange between system and universe; positive when energy is received by the system.

$dV$ = volume change of the system during process.

$\mu_\gamma$ = chemical potential of component $\gamma$.

$dn_\gamma$ = change in number of molecules per unit volume of substance $\gamma$.

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The Gibbs equation was developed for systems at equilibrium. Systems undergoing spontaneous changes are not at equilibrium, but it is postulated in nonequilibrium thermodynamics that, in such systems, there exists at every point a state of <u>local</u> equilibrium for which the local entropy is given by the Gibbs equation.[41,48] This assumption restricts discussion to systems that may be described in terms of macroscopic thermodynamics and hydrodynamics, without explicit reference to molecular concepts.

## C. ENTROPY PRODUCTION

The total entropy change of a system, dS, is usually examined in terms of the change per unit time (dS/dt). In conformity with Section VII, A, above, $(dS/dt) = [(d_eS/dt) + (d_iS/dt)]$, the total entropy change per unit time being the sum of the entropy exchanged with the environment per unit time, and the entropy produced in the system per unit time.

The whole volume of a system is the sum of many very small volume elements. Since entropy is an extensive quantity, the internal entropy production in the entire system is the sum of the internal entropy production in all these volume elements. In each volume element, the entropy production is called the "local entropy production"[26,41,48] and is given the symbol $\sigma$. The local entropy production can be broken down into three parts: (i) production of "pure" entropy, (ii) production of entropy by diffusion of one or more substances present, and (iii) production of entropy because of chemical reactions occurring in the system. (There is imposed on the system we are now considering the condition of mechanical equilibrium. This means there is no bulk flow of liquids; entropy production of moving amoebae, for example, cannot be described by these equations. This restriction is lifted in Section VI, A.)

Each of these three components of entropy production is a product of two terms, one a flow, the other a force:

    1) Production of "pure" entropy = heat flow x gradient of a temperature function,

    2) Production of entropy by diffusion process = flow of matter by diffusion x gradient of a function of chemical potential. Note that diffusion flow is not the same as bulk flow. In diffusion, the diffusing substance moves relative to all other substances in the element of volume considered. In bulk flow, which is zero in the present discussion, all substances in an element of volume move in concert in the same direction; there is no movement of one substance relative to the others present.

    3) Production of chemical reaction entropy = rate of chemical reaction x a function of the affinity of the reaction.

To summarize these equations we may write

$$\sigma = \sum_{i=1}^{n} J_i X_i \qquad i = 1, 2, \ldots, n \qquad (9)$$

Once a flow is described, the primary force associated with it is fixed by certain thermodynamical and mathematical requirements. Such flows and forces are said to be "conjugate"; $J_i$ is the flow conjugate to the force $X_i$.

Because entropy is a defined quantity, not directly determinable experimentally, this equation is not often used directly. The primary value of the local entropy production is to establish the flows and forces involved in the irreversible processes occurring in the system of interest. The flows and forces are also connected by the Linear Laws, which are the equations needed for experimental work.

## D. LINEAR LAWS

Consider the thermocouple. This device measures a potential difference to determine the temperature difference between the junctions. This might seem strange, for a potential difference between two points is association, by Ohm's Law, with flow of electricity between the points and Ohm's Law says nothing of temperature differences. Furthermore, Fourier's Law relates temperature difference to heat flow and does not consider electrical potential differences.

The conclusion must be that, because there is a temperature difference there is a heat flow; and because there is a measurable potential difference there is an electric current.[26]* Furthermore, it has been observed that if one forces an electric current (d.c.) through a thermocouple whose junctions are at the same temperature (e.g., by using a battery), the junctions do not remain at the same temperature; one becomes warmer and the other cooler than their common initial temperature.

The interrelationship of these four quantities: heat flow, electric current, temperature difference, and potential difference can be expressed by these equations:

$$\text{Heat flow} = L_{11} \times \frac{\text{temperature difference}}{(\text{average temperature})^2} + L_{12} \times \frac{\text{potential difference}}{\text{average temperature}}$$

$$(10)$$

$$\text{Electric current} = L_{21} \times \frac{\text{temperature difference}}{(\text{average temperature})^2} + L_{22} \times \frac{\text{potential difference}}{\text{average temperature}}$$

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* Chapter 8.

These equations assert the following:

1) Each flow in the system is a function of both the forces operating in the system. The forces both involve the average temperature: one is $(\Delta T/T^2)$, the other $(\Delta\phi/T)$. These forces meet the requirement of being those conjugate to the flow to which they are primarily related (see Section VII, C).

2) The dependence of the flow on each force is linear, that is, it depends on the first power of the force.

3) There is a proportionality constant connecting each flow with each of the forces driving it.

These equations can be summarized in the following mathematical form:

$$J_i = \sum_{k=1}^{n} L_{ik}X_k, \qquad k,i = 1,2,\ldots,n \qquad (11)$$

Equations such as those in the example are based on experimental observations. They are therefore referred to as phenomenological relations. They are also called Linear Laws because they involve only the first power of the forces.

## E. PHENOMENOLOGICAL COEFFICIENTS AND THE ONSAGER RECIPROCAL RELATIONS

The coefficients in the equations in Section VII, D, designated phenomenological coefficients, must be determined experimentally; it is a postulate of nonequilibrium thermodynamics[26,48] that they are constant over the range where the Linear Laws hold. In these equations the coefficient designated $L_{11}$ is commonly called the heat conductivity of the metal junction and that designated $L_{22}$ is commonly called the electrical conductivity of the metal junction. These quantities are characteristics of the system and are independent of each other. The other two coefficients, $L_{12}$ and $L_{21}$, are called "cross-coefficients." They are independent of both $L_{11}$ and $L_{22}$. They are not independent of each other; in fact $L_{12} = L_{21}$ in all cases where such Linear Laws describe a system. This was proved by Onsager;[49,50] consequently, this is known as the Onsager Reciprocal Relation.

## F. DEGREE OF ADVANCEMENT

This concept applies to any process, chemical or physical.[47] It is a number between zero and one that expresses how far the process has gone from inception to completion, completion generally being a point of no further net changes. The accepted symbol for the quantity is $\xi$. In other terminology one can say that all processes advance along a "reaction coordinate." The quantity $\xi$ measures progress along this coordinate.

Consider the simple chemical process of carbon dioxide reacting with water to form carbonic acid. The reaction can be written as follows:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \tag{12}$$

If one abruptly exposes a previously protected body of chemically pure water to the atmosphere, which normally contains a low concentration of $CO_2$, the degree of advancement of this reaction as it is written above is zero at the instant of exposure. As carbon dioxide dissolves from the atmosphere and forms carbonic acid the degree of advancement reaches unity as a steady state is established under the prevailing conditions of temperature and carbon dioxide partial pressure. This steady state is commonly called the equilibrium value under prevailing temperature and pressure constraints. It changes if the total atmospheric pressure changes, even if the partial pressure of $CO_2$ is not altered. The change in degree of advancement per unit of time is the reaction velocity:

$$\frac{d\xi}{dt} = v \tag{13}$$

## G. AFFINITY

Let us arrange it so that in a thermodynamic system there are conditions of constant temperature and constant pressure, and that there is occurring a spontaneous chemical reaction. Since the chemical reaction is occurring spontaneously, it is irreversible. At any point in time during the course of this reaction, there will have been released to the surroundings a quantity of heat proportional to the change in the degree of advancement of the reaction. To put this in the form of an equation we write:

$$dQ' = Ad\xi \geq 0 \tag{14}$$

in which the proportionality constant, A, is called the affinity.[47] The heat produced by the irreversible reaction is a direct measure of the entropy produced.

We will define affinity in words as a measure of the tendency for the irreversible reaction to occur. As a mathematical equation we write its definition:

$$A = - \Sigma_\gamma \nu_\gamma \mu_\gamma \qquad \gamma = 1, 2, \ldots, n \tag{15}$$

in which

$\nu_\gamma$ = the stoichiometric coefficient in the reaction equation of the substance $\gamma$.

$\mu_\gamma$ = the chemical potential of the substance $\gamma$.

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For example, in the chemical reaction

$$N_2 + 3H_2 \rightleftharpoons 2NH_2 \qquad (16)$$

the stoichiometric coefficient of nitrogen is -1, of hydrogen is -3, and of ammonia, +2. Therefore, the affinity of the reaction is written in this manner:

$$A = -\Sigma(-\mu_{N_2} - 3\mu_{H_2} + 2\mu_{NH_3}) = \mu_{N_2} + 3\mu_{H_2} - 2\mu_{NH_3} \qquad (17)$$

Certain properties of this quantity, affinity, are stated here. For more detailed information consult the book by Prigogine and Defay.[47]*

1) Affinity is a function of state.

2) Affinity always has the same algebraic sign as the velocity of the reaction.

3) If the affinity is zero the rate of reaction is zero, i.e., the system is in equilibrium.

4) It is possible for the affinity of the reaction to be very high and for the rate of the reaction to be zero; this is a case of false equilibrium. For example, a mixture of hydrogen and oxygen has a very high affinity but will not react to form water unless reaction is initiated by a spark or by a catalyst.

H.  THE COUPLING OF REACTIONS

If there are several reactions going on at the same time within a system, the total entropy produced per unit time and per unit volume is the sum of the entropy produced per unit time and per unit volume of the individual reactions:[41,47]

$$P \equiv \frac{d_i S}{dt} = \frac{1}{T} \Sigma_\rho A_\rho v_\rho \geq 0 \qquad \rho = 1, 2, \ldots, r \qquad (18)$$

in which the subscript $\rho$ refers to each of the reactions involved and can assume the value $1, 2, \ldots, r$. The symbol P is often used in the literature to designate the quantity $(d_i S/dt)$. This equation is one specific form of the general equation for entropy production given in equation (9). The present example involves only chemical reactions. Thus, in a system where two reactions occur in a "local" region it is possible to have
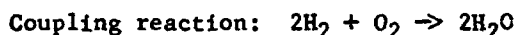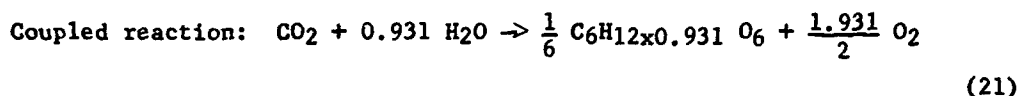
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* Chapter 3.

$$A_1V_1 < 0 \quad , \quad A_2V_2 > 0 \tag{19}$$

provided that

$$(A_1V_1 + A_2V_2) \geq 0 \tag{20}$$

The first reaction is then celled the coupled reaction and the second the coupling reaction. Such reactions involve an entity common to both. Thus, because of thermodynamic coupling, the coupled reaction may proceed in a direction opposite to that dictated by its affinity. An example may be seen in the synthesis of carbohydrate coupled to the combustion of elemental hydrogen in Bacillus pycnoticus.[51] The chemical reactions involved are these:

Coupled reaction: $CO_2 + 0.931 \ H_2O \rightarrow \frac{1}{6} C_6H_{12x0.931} O_6 + \frac{1.931}{2} O_2$

$$\tag{21}$$

Coupling reaction: $2H_2 + O_2 \rightarrow 2H_2O$

The coupled reaction [indicated by subscript 1 in equation (20)] is so designated because a carbohydrate similar in formula weight to glucose was observed to accumulate with time in the bacterium when it grew in a solution of $H_2$, $O_2$, and $CO_2$. Hydrogen plays the role of metabolite, for the mass present decreases as a result of combustion with oxygen. The hydrogen combustion is the coupling reaction [indicated by subscript 2 in equation (20)].

The affinities of the reactions, and their velocities, were found to be

$$A_1 = -105,100 \ cal \qquad V_1 = 0.548 \times 10^{-3}$$
$$\tag{22}$$
$$A_2 = +108,500 \ cal \qquad V_2 = 2.377 \times 10^{-3}$$

If inequality equation (20) holds there is an upper limit on $V_1$:

$$V_1 \leq \frac{|A_2|V_2}{|A_1|} \tag{23}$$

The absolute values of affinities are used because the sign indicates only the direction of heat flow with regard to the system.

Substituting the experimental values in equation (22) verifies equation (23):

$$0.548 \times 10^{-3} < \frac{108,500}{105,100} \times 2.377 \times 10^{-3} \tag{24}$$

This abstract of van Rysselberghe's work glosses over most of the mathematics but violates neither its thermodynamical nor its mathematical verities.

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